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Chapter 7

Plasma biomarkers for Acute Respiratory Distress Syndrome: a systematic review and meta-analysis

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ABSTRACT

Introduction Numerous studies have focused on biomarkers for acute lung injury and acute respiratory distress syndrome (ARDS). Although several biomarkers have been identified, their relative performance is unclear. We aim to provide a quantitative overview of plasma-derived biomarkers associated with ARDS diagnosis or mortality.

Data sources MEDLINE (inception to January 2012) and personal databases.

Study Selection English-language studies on plasma biomarkers associated with ARDS diagnosis or mortality.

Data extraction Demographic variables, plasma levels of biomarker, statistical data, ARDS occurrence and mortality rates were retrieved. The methodological quality was assessed with the QUADAS score. Clinical outcomes included: 1) diagnosis of ARDS in the at risk population and 2) mortality in ARDS patients. For each biomarker pooled odds ratios (OR) for clinical outcome were calculated by meta-analysis, and biomarkers were ranked according to pooled OR.

Data synthesis Fifty-four studies appeared eligible for meta-analysis, together including 3753 patients. We identified 20 biomarkers for diagnosis of ARDS in the at risk population and 19 biomarkers for mortality of ARDS patients. The biomarkers most strongly associated with ARDS diagnosis in the at risk population, when increased, were Krebs von der Lungen-6 (KL-6, OR[95%CI], 6.1[3.0-12.1]), lactate dehydrogenase (5.7[1.7-19.1]), soluble receptor for advanced glycation endproducts (3.5 [1.7-7.2]) and von Willebrand Factor (3.1[2.0-5.2]). The biomarkers most strongly associated with ARDS mortality, when increased, were Interleukin (IL)-4 (18.0[6.0-54.2]), IL-2 (11.8[4.3-32.2]), angiopoietin-2 (6.4 [1.3-30.4]) and KL-6 (5.1[3.0-12.2]). Decreased levels of Protein C were associated with increased odds for ARDS diagnosis and mortality.

Conclusion This meta-analysis provides a unique ranking of plasma biomarkers according to their strength of association with ARDS diagnosis or ARDS mortality. The relative performance of biomarkers among studies shown in this ranking may help to improve ARDS diagnosis and outcome prediction.

INTRODUCTION

The acute respiratory distress syndrome (ARDS) is a clinical syndrome, characterized by tachypnea, severe hypoxemia, decreased respiratory compliance and lung tissue damage evident from chest X-ray [1]. Although diffuse alveolar damage forms the core pathological process [2], diagnosis of ARDS and its milder form acute lung injury (ALI) are based on clinical characterization. These clinical characteristics are formalized in the American European Consensus Conference (AECC) criteria [3]. However, the accuracy of the AECC criteria has been questioned since their publication in 1994 [4-6], hampering diagnosis of ALI/ARDS.

In addition to a 2012 revision of the clinical criteria for ALI/ARDS, known as the Berlin definition [7], numerous studies have focused on the identification of biomarkers to improve classification and definition of ARDS. According to the 2001 NIH definition, a biomarker is "*a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention*" [8]. Biomarkers reflect pathophysiological mechanisms and as such may help to recognize ARDS. Combining existing clinical definitions with reliable biomarkers may therefore enhance the diagnosis of ARDS. Besides recognition of ARDS, biomarkers may contribute to risk stratification and prediction of outcome or serve as surrogate endpoints to monitor intervention [9].

The proposed advantages of biomarkers [9], together with the limited reliability and validity of the AECC criteria [5,6], have spurred the search for reliable ARDS biomarkers in the last two decades. This search has resulted in a large spectrum of ARDS biomarkers, ranging from inflammatory mediators to tissue degradation products, and from plasma-derived biomarkers to genetic polymorphisms. Recent reviews have provided excellent narrative overviews of available biomarker studies and their relation to the pathophysiology of ARDS [9,10]. However, a quantitative review or comparison of the performance of the several biomarkers studied is lacking.

In the current study we conducted a systematic review and meta-analysis of all studies on plasma biomarkers associated with either diagnosis of ARDS in the at risk population or ARDS-related mortality. This study provides a quantitative overview of all plasma-derived biomarkers of ARDS studied thus far, and may help to establish reliable ARDS biomarkers.

METHODS

Data source & Study selection

For this study a systematic literature search in MEDLINE and personal databases was performed to identify all studies reporting on plasma biomarker in patients with or at risk for ARDS. Details

of the search strategy are outlined in the Supplementary Information. All studies obtained by this search were evaluated for eligibility by two independent researchers (M.L.T. and J.A.). In case of disagreement a third investigator (A.B.J.G.) was consulted. Eligibility of a study for the meta-analysis was based on the following selection criteria: report of original research, inclusion of adults with or at risk for ARDS, report of plasma concentration (absolute values) of a biomarker related to a clinical outcome (occurrence and mortality of ARDS in the study population), description of demographic variables, and written in English. Studies were excluded if they were the only study reporting on a specific biomarker.

Data extraction and quality assesment

In a standardized fashion the following data were retrieved (by M.L.T. and J.A) from the included studies: data on ARDS etiology, sample size of the subgroups, mean or median plasma level of the biomarker (per subgroup), and P-value of the statistical test that was used to compare the subgroups. When in an included study a biomarker was measured more than once, only the baseline measurement (i.e. the first measurement provided) was extracted. In addition, demographic variables (age, sex, total number of participants), the diagnostic criteria used for ARDS diagnosis, mortality and the moment of biomarker sampling were retrieved.

All studies were assessed on methodological quality according to the QUADAS score, which varies between 0 and 14 [11] (Supplementary Digital Content). The meta-analysis and reporting of the data were performed according to the Proposal for Reporting Meta-analysis of Observational Studies in Epidemiology [12].

Data synthesis and analysis

Two types of clinical outcome were considered in this study: 1) diagnosis of ARDS in the at risk population and 2) mortality of patients in the ARDS population. To analyze the strength of association of a biomarker with clinical outcome, we extracted plasma levels of the biomarker from different subgroups: ARDS patients versus critically ill non-ARDS patients (for association with ARDS diagnosis in the at risk population), or survivors versus non-survivors in a cohort of ARDS patients (for association with ARDS mortality among ARDS patients). Studies comparing ARDS patients with healthy controls were excluded for reasons of comparability. The authors' definition of ARDS as given in the articles was taken.

Statistics

Meta-analyses were performed with Comprehensive Meta-Analysis version 2.2 (Biostat Inc, Englewood, NJ, US) using a randomized model. To determine the strength of association of

a specific biomarker with clinical outcome the following statistical procedure was performed: For each study we calculated the standardized difference of the mean in biomarker levels between the subgroups relevant for clinical outcome (subgroups: No ARDS vs. ARDS, Survivors vs. Non-survivors). The standardized difference of the mean is based on the exact P-value and the population size, and allows subsequent calculation of the odds ratio (OR) of the specific biomarker for clinical outcome. Subsequently the pooled OR of different studies on a biomarker was calculated. Data are presented as OR with 95% confidence interval (OR [95% CI]). Forest plots are provided for biomarkers of which five or more studies are included in the meta-analysis. Biomarkers were ranked according to OR and statistical significance.

I^2 and Tau^2 statistics were performed to assess heterogeneity among studies. Funnel plots were created to evaluate publication bias, which was further analyzed with Egger's regression test [13], Duval and Tweedie's trim and fill [14] and Orwin's fail-safe N test [15]. For Orwin's fail-safe N test the clinically trivial OR value was arbitrarily set at 1.25 for OR>1 or at 0.8 for OR<1.

With SPSS (SPSS 20, IBM Corporation, Armonk, NY, US) the weighted average of each biomarker was calculated for the subgroups mentioned before, based on the average value provided in a study (mean and median were considered here as equal, i.e. value most representative for a specific population) and the size of the study population. Weighted averages are presented as mean ± standard deviation and range. Exact P-values are given unless <0.001. A P-value <0.05 was considered statistically significant, except for Egger's regression test in which a P-value <0.10 was considered statistically significant.

RESULTS

Literature search

The MEDLINE search yielded 509 articles, whereas the search of personal databases yielded 253 articles. From the initial 762 studies, 672 studies were excluded because of: duplicate studies (n=243), other language than English (n=49), lack of focus on ARDS (n=73), in vitro/animal studies (n=86), no original research (reviews, editorials or case-reports, n=49), pediatric studies (n=22), no biomarker measurement in plasma (n=61), no relation of biomarker with occurrence or mortality of ARDS as clinical outcome (n=56), use of biomarker for treatment monitoring (n=18) or insufficient data (absolute plasma concentrations or P-values, n=13). From one study no full text copy was available despite attempts to contact the authors [16]. After removal of these studies, 91 studies were found eligible for data retrieval. Of these 91 studies, 37 studies were the only study that reported on a specific biomarker, precluding meta-analysis. The remaining 54 articles [17-70] were used for meta-analysis (Figure 1).

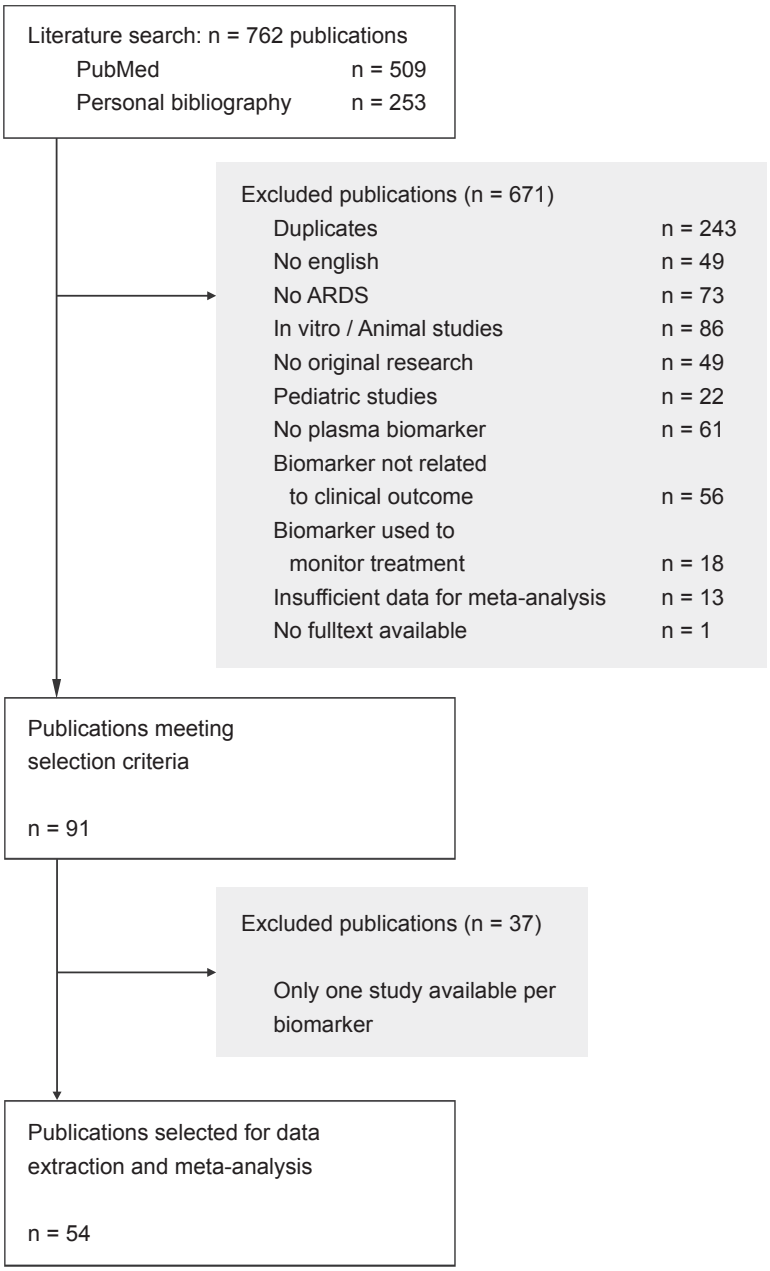


Figure 1 – Flow chart of literature search. ARDS = acute respiratory distress syndrome.

Study characteristics & Quality Assessment

Demographic variables of the included studies are presented in Supplementary Table 1. In total 3753 patients were included. Only adult studies were considered, resulting in an age range from 33 to 78 years. In the majority of studies, ALI or ARDS was diagnosed according to the AECC criteria (41/54). Alternative criteria involved the lung injury score (LIS, 7/54) [71], the ratio of protein in edema fluid / protein in plasma (3/54), the so-called Fowler criteria (3/54) [72], or non-formalized clinical criteria (5/54). The mortality in the selected studies ranged from 0 to 70%. The time point on which plasma samples were taken for biomarker detection varied from 0 to 72 hours/days after ICU admission (Supplementary Table 1).

Supplementary Table 2 provides details on the ARDS etiology from the included studies. Causes of ARDS most frequently encountered were sepsis (42/54 studies), pneumonia (35/54 studies), trauma/major surgery (24/54 studies), aspiration (23/54 studies) and multiple transfusions (14/54 studies). The majority of studies had an ARDS patient population that was characterized by multiple etiologies. Several studies reported data on more than one biomarker. Moreover, a number of studies provided data on both clinical outcomes, i.e. diagnosis of ARDS in the at risk population and mortality of patients with ARDS. The median quality of the included publications as assessed by the QUADAS score was 13 (range 11–14, Supplementary Table 1).

Biomarkers associated with ARDS diagnosis in the at risk population

We performed meta-analyses on 20 different plasma biomarkers that were associated with the diagnosis ARDS in the at risk population (Table 1). For four of these biomarkers (von Willbrand factor (vWF), soluble receptor for advanced glycation end products (sRAGE), interleukin (IL)-6, tumor necrosis factor-α (TNF-α)) $\geq$5 studies per biomarker were identified. For the other biomarkers only two to four studies per biomarker were found. The pooled ORs for the presence of ARDS in the at risk population are summarized and ranked in Table 1. The highest pooled ORs were observed for Krebs von den Lungen-6 (KL-6), lactate dehydrogenase (LDH), sRAGE, vWF and IL-8. Figure 2 shows forest plots of the biomarkers with data available of at least 5 studies (Figure 2). For almost all biomarkers, an elevated plasma concentration was associated with increased odds for ARDS (OR>1). Otherwise, decreased plasma concentrations of transferrin and protein C were associated with increased odds (OR<1) for ARDS. For Clara Cell-16, C-Reactive Protein and ICAM-1 no significant OR for ARDS diagnosis was found. Among biomarkers with significant OR for ARDS diagnosis, no significant heterogeneity was observed (Table 1).

Table 1 – Biomarkers associated with ARDS diagnosis in the at-risk population

Biomarker	Nr. of studies	Nr. of patients	OR (95% CI)	Heterogeneity (%)		
				I^2	Tau^2	P-value
KL-6	4 [17-20]	137	6.06 [3.04-12.1]	<0.001	<0.001	0.41
LDH	2 [21,22]	53	5.67 [1.69-19.1]	<0.001	<0.001	0.99
sRAGE	5 [17,18,25,30,31]	317	3.48 [1.69-7.15]	51.05	0.328	0.09
vWF	5 [23-27]	385	3.22 [2.01-5.17]	19.54	0.060	0.29
IL-8	4 [25,32-34]	329	3.21 [1.41-7.29]	58.29	0.343	0.07
Ang-2	2 [25,26]	275	2.98 [1.16-7.65]	68.15	0.325	0.08
sP-selectin	2 [28,29]	107	2.79 [1.17-6.65]	<0.001	<0.001	0.40
SP-D	3 [18,25,35]	249	2.77 [1.20-6.39]	46.08	0.263	0.16
sE-selectin	2 [22,29]	109	2.51 [1.03-6.13]	15.47	0.096	0.28
TNF-α	5 [25,32,34,36,38]	434	2.45 [1.33-4.51]	55.84	0.246	0.06
IL-6	5 [25,32-34,36,37]	407	2.37 [1.32-4.26]	43.71	0.203	0.11
IL-10	2 [25,32]	304	2.22 [1.14-4.34]	57.84	0.137	0.12
PAI-1	3 [25,39,40]	1121	1.78 [1.32-2.39]	<0.001	<0.001	0.83
IL-1β	2 [32,36]	178	1.77 [1.01-3.10]	<0.001	<0.001	0.42
CC-16	3 [17,25,41]	260	2.54 [0.84-7.92]	71.67	0.712	0.03
CRP	3 [22,32,37]	156	2.40 [0.71-8.17]	58.74	0.693	0.12
ICAM-1*	2 [25,42]	259	1.84 [0.68-4.98]	67.47	0.364	0.08
Transferrin	2 [43,44]	85	0.13 [0.03-0.52]	47.09	0.479	0.17
Protein C*	2 [39,45]	945	0.49 [0.32-0.64]	<0.001	<0.001	0.52
sL-selectin	2 [29,37]	99	0.54 [0.02-13.45]	89.56	4.827	<0.01

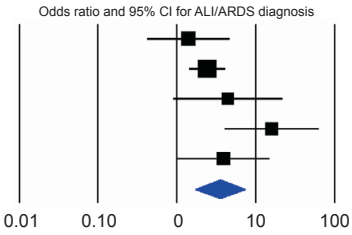
*Included data are reported by the same research group.
Ang-2 = angiopoietin-2, CC-16 = clara cell protein-16, CRP = c-reactive protein, ICAM-1 = intercellular adhesion molecule-1, IL = interleukin, KL-6 = Krebs von den Lungen-6, LDH = lactate dehydrogenase, PAI-1 = plasminogen activator inhibitor-1, sE-selectin = soluble E-selectin, sL-selectin = soluble L-selectin, sP-selectin = soluble P-selectin, SP-D = surfactant protein-D, sRAGE = soluble receptor for advanced glycation end products, TNF-α = tumor necrosis factor-α, vWF = von Willebrand factor. Pooled ORs are calculated on the basis of randomized effects.

Biomarkers associated with ARDS mortality

We performed meta-analyses on 19 biomarkers reported to be associated with ARDS mortality. For four of these biomarkers (IL-1β, TNF-α, IL-8, IL-6) ≥5 studies per biomarker were identified. For the remaining biomarkers two to four studies per biomarker were found (Table 2). The pooled ORs for ARDS mortality are ranked in Table 2. Biomarkers with the highest ORs for mortality were IL-4, IL-2, angiopoietin (Ang)-2 and KL-6. Figure 3 shows forest plots of the pooled OR of IL-1β, TNF-α, IL-8, IL-6 for ARDS mortality. Protein C was the only biomarker of which decreased plasma levels were associated with increased odds for ARDS mortality. For procalcitonin, plasminogen activator inhibitor (PAI)-1, IL-10, Clara Cell-16, sRAGE and SP-A no significant OR for ARDS mortality was found.

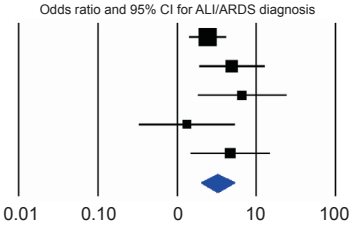
sRAGE

Study name	Sample size	Odds ratio (95% CI)	P-value
Determann '10	36	1.4 (0.4 - 4.6)	0.59
Fremont '10	192	2.4 (1.4 - 4.1)	<0.01
Determann '09	22	4.4 (0.9 - 21.6)	0.07
Jabaudon '08	34	15.9 (4.0 - 63.8)	<0.01
Uchida '06	33	3.9 (1.0 - 15.2)	0.05
Total	317	3.5 (1.7 - 7.2)	<0.01



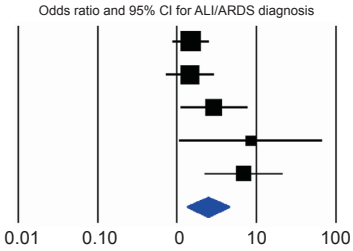
vWF

Study name	Sample size	Odds ratio (95% CI)	P-value
Fremont '10	192	2.4 (1.4 - 4.1)	<0.01
Van der Heijden '08	83	4.9 (1.9 - 12.6)	<0.01
Siemiatkowski '00	36	6.6 (1.8 - 24.5)	0.01
Moss '95	29	1.3 (0.3 - 5.3)	0.40
Rubin '90	45	4.7 (1.5 - 15.1)	0.01
Total	385	3.2 (2.0 - 5.2)	<0.01



TNF-α

Study name	Sample size	Odds ratio (95% CI)	P-value
Fremont '10	192	1.5 (0.9 - 2.5)	0.14
Lee '10	112	1.5 (0.7 - 2.9)	0.29
Bauer '00	66	2.9 (1.1 - 7.7)	0.03
Cholett-Martin '96	14	8.5 (1.1 - 67.4)	0.04
Roten '90	50	6.9 (2.1 - 21.5)	<0.01
Total	434	2.5 (1.3 - 4.5)	<0.01



IL-6

Study name	Sample size	Odds ratio (95% CI)	P-value
Fremont '10	192	2.1 (1.3 - 3.6)	0.005
Lee '10	112	2.1 (1.1 - 4.3)	0.03
Takala '01	11	35.5 (2.6 - 487.0)	0.01
Bauer '00	66	1.0 (0.4 - 2.6)	1.00
Hensel '98	17	3.6 (0.6 - 21.4)	0.16
Cholett-Martin '96	14	8.5 (1.1 - 67.4)	0.04
Total	220	2.4 (1.3 - 4.3)	<0.01

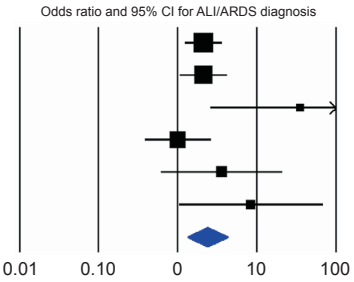
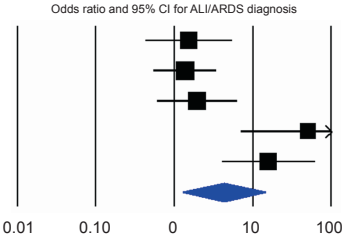


Figure 2 – Odds ratios for ARDS diagnosis. Forest plots of pooled odds ratios for the strength of association between biomarkers and the development of ARDS in the at risk population. IL = interleukin, sRAGE = soluble receptor for advanced glycation endproducts, TNF-α = tumor necrosis factor-α, vWF = von Willebrand factor.

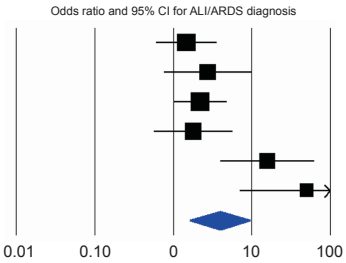
IL-1β

Study name	Sample size	Odds ratio (95% CI)	P-value
Lee '10	44	1.5 (0.4 - 5.5)	0.52
Lin '10	63	1.4 (0.6 - 3.4)	0.50
Bauer '00	46	1.9 (0.6 - 6.3)	0.27
Medurii '95	21	50.3 (7.0 - 358.5)	<0.01
Headley '95	34	15.8 (4.0 - 63.3)	<0.01
Total	208	4.3 (1.3 - 14.4)	<0.01



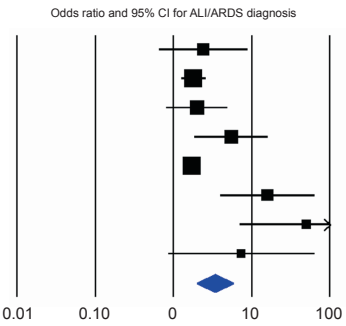
TNF-α

Study name	Sample size	Odds ratio (95% CI)	P-value
Lin '10	63	1.5 (0.6 - 3.7)	0.40
Lee '10	44	2.8 (0.8 - 10.2)	0.12
Chen '09	88	2.2 (1.0 - 4.8)	0.05
Bauer '00	46	1.8 (0.6 - 5.8)	0.32
Headley '95	34	15.8 (4.0 - 63.3)	<0.01
Meduri '95	21	50.3 (7.0 - 58.5)	<0.01
Total	296	3.9 (1.6 - 9.4)	<0.01



IL-8

Study name	Sample size	Odds ratio (95% CI)	P-value
Lee '10	44	2.4 (0.7 - 8.8)	0.18
Ware '10	528	1.8 (1.3 - 2.5)	<0.01
Lin '10	63	2.0 (0.8 - 5.0)	0.14
McClintock '08	50	5.5 (1.9 - 16.0)	<0.01
Parsons '05	780	1.7 (1.3 - 2.2)	<0.01
Headley '95	34	15.8 (4.0 - 63.3)	<0.01
Meduri '95	21	50.3 (7.0 - 58.5)	<0.01
Groeneveld '95	13	7.3 (0.8 - 64.1)	0.07
Total	1533	3.4 (2.0 - 5.7)	<0.01



IL-6

Study name	Sample size	Odds ratio (95% CI)	P-value
Nakamura '11	20	1.4 (0.3 - 6.8)	0.69
Lee '10	44	4.6 (1.2 - 17.0)	0.18
Ware '10	521	1.7 (1.2 - 2.4)	<0.01
Parsons '05	781	1.7 (1.3 - 2.2)	<0.01
Bauer '00	46	1.1 (0.3 - 3.6)	0.32
Headley '95	34	15.8 (4.0 - 63.3)	<0.01
Meduri '95	21	50.3 (7.0 - 358.5)	<0.01
Groeneveld '95	13	64.2 (4.9 - 848.6)	<0.01
Total	1480	3.4 (1.8 - 6.3)	<0.01

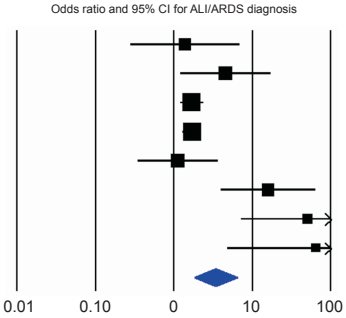


Figure 3 – Odds ratios for ARDS mortality. Forest plots of pooled odds ratios for the strength of association between biomarkers and mortality in ARDS patients. IL = interleukin, TNF-α = tumor necrosis factor-α.

Table 2 – Biomarkers associated with ARDS mortality

Biomarker	Nr. of studies	Nr. of patients	OR [95% CI]	Heterogeneity (%)		
				I^2	Tau^2	P-value
IL-4*	2 [46,47]	55	18.0 [5.96-54.2]	<0.001	<0.001	0.77
IL-2	3 [46-48]	62	11.8 [4.32-32.2]	<0.001	<0.001	0.55
Ang-2	2 [52,53]	227	6.36 [1.33-30.35]	58.75	0.825	0.12
KL-6	4 [20,49-51]	136	4.29 [1.84-9.99]	38.32	0.285	0.18
IL-1β	5 [32,36,46,47,54]	208	4.28 [1.28-14.37]	77.70	1.441	<0.01
TNF-α	6 [32,36,46,47,54,55]	296	3.91 [1.62-9.44]	70.76	0.816	<0.01
IL-6	8 [32,36,46,47,51,60,62,63]	1480	3.38 [1.81-6.31]	77.158	0.434	<0.01
IL-8	8 [32,36,46,54,60-63]	1533	3.35 [1.96-5.71]	72.58	0.310	<0.01
vWF	4 [27,60,64,65]	1146	2.60 [1.44-4.68]	72.92	0.203	0.01
CRP	3 [32,54,59]	284	2.29 [1.47-3.57]	<0.001	<0.001	0.42
ICAM-1	3 [60,61,67]	629	1.92 [1.40-2.62]	<0.001	<0.001	0.64
SP-D*	2 [60,68]	1081	1.53 [1.21-1.94]	<0.001	<0.001	0.80
PCT	2 [56,57]	74	7.56 [0.24-234.6]	88.15	5.445	<0.01
PAI-1*	2 [60,66]	499	3.87 [0.61-24.42]	77.96	1.436	0.03
IL-10	2 [32,62]	637	2.80 [0.91-8.68]	66.23	0.487	0.08
CC-16	2 [41,58]	98	2.11 [0.73-6.11]	39.64	0.256	0.20
sRAGE	3 [30,51,67]	756	2.54 [0.75-8.63]	73.53	0.830	0.02
SP-A*	2 [68,69]	603	1.14 [0.60-3.54]	58.73	0.277	0.12
Protein C	4 [60,61,70,71]	668	0.35 [0.17-0.72]	65.09	0.343	0.04

*Included data are reported by the same research group.
Ang-2 = angiopoietin-2, CC-16 = clara cell protein-16, CRP = c-reactive protein, ICAM-1 = intercellular adhesion molecule-1, IL = interleukin, KL-6 = Krebs von den Lungen-6, LDH = lactate dehydrogenase, PAI-1 = plasminogen activator inhibitor-1, PCT = procalcitonin, SP-A = surfactant protein-A, SP-D = surfactant protein-D, sRAGE = soluble receptor for advanced glycation end products, TNF-α = tumor necrosis factor-α, vWF = von Willebrand factor. Pooled ORs are calculated on the basis of randomized effects.

For six biomarkers (IL-1β, TNF-α, IL-8, vWF, IL-6 and protein C) significant heterogeneity was observed. Evaluating heterogeneity, we found that it was predominantly caused by a limited number of studies, which reported extreme ORs [27,46,47,63]. Excluding the studies with extreme ORs (arbitrarily chosen as OR < 0.10 or OR > 10.0) diminished heterogeneity, while the pooled OR remained significant for all biomarkers except IL-1β (Table 3).

Weighted average of biomarkers per subgroup

To provide insight in the absolute plasma concentrations of the biomarkers included in this meta-analysis, we calculated the mean values of the plasma concentration provided in each study. Supplementary Table 3 provides the weighted average of each biomarker in patients at risk versus patients with AL/ARDS, while Supplementary Table 4 provides the weighted averages of each biomarker in survivors versus non-survivors.

Table 3 – Heterogeneity analysis of biomarkers associated with ARDS mortality

Biomarker	Removed studies	Nr. of patients removed	Remaining studies	Nr. of patients remaining	Corrected OR [95% CI]	Corrected heterogeneity (%)		
						I^2	Tau^2	P-value
IL-1β	2 [46,47]	55	3 [32,36,54]	153	1.55 [0.83-2.90]	<0.001	<0.001	0.89
TNF-α	2 [46,47]	55	4 [32,36,54,55]	141	1.96 [1.20-3.20]	<0.001	<0.001	0.86
IL-8	3 [46,47,63]	68	5 [32,54,60-62]	1465	1.87 [1.48-2.36]	11.33	0.009	0.34
vWF	1 [27]	36	3 [60,64,65 *]	1110	1.98 [1.46-2.68]	31.12	0.023	0.23
IL-6	3 [46,47,63]	68	5 [32,36,51,60,62]	1412	1.71 [1.39-2.10]	<0.001	<0.001	0.61

*All studies included in this analysis are reported by the same research group.
IL = interleukin, OR = odds ratio, sRAGE = soluble receptor for advanced glycation end products, TNF-α = tumor necrosis factor-α, vWF = von Willebrand factor. Pooled ORs are calculated on the basis of randomized effects.

Evaluation of publication bias

Egger's regression test revealed that for a number of biomarkers there was a significant association between effect size and precision – among biomarkers associated with ARDS diagnosis a P-value <0.10 was found for IL-8 and TNF-α, while among biomarkers associated with mortality a P-value <0.10 was found for IL-1β, TNF-α, IL-8 and IL-6. However, when we adjusted for possible publication bias by Duval & Tweedie's trim and fill, the OR remained statistically significant for all biomarkers. The relatively large number of missing studies required to bring these ORs to a clinically trivial value, as calculated with Orwin's fail-safe N test, suggest that the chance that the entire effect observed in our study is a matter of bias, is small (Supplementary Table 5). Supplementary Figures 1 and 2 show funnel plots for the meta-analyses in which ≥5 studies per biomarker were identified.

DISCUSSION

In the current study we performed a meta-analysis of publications reporting on plasma biomarkers for their strength of association with ARDS in the at risk population or ARDS mortality among ARDS patients. Searching MEDLINE and personal databases we identified 54 publications, together including 3753 patients with or at risk for ARDS. We show that increased plasma levels of KL-6, LDH, sRAGE and vWF are most strongly associated with ARDS diagnosis in the at risk population, whereas the strongest association with ARDS mortality was found for IL-4, IL-2, Ang-2 and KL-6. Increased plasma levels of transferrin and protein C were associated with decreased odds for both clinical outcomes. This unique ranking of ARDS plasma biomarker according to their strength of association may help to establish reliable ARDS biomarkers.

This is the first study providing a quantitative overview of biomarker research in ARDS performed thus far. Collecting data from almost four thousand patients we ranked reported biomarkers according to their pooled OR for ARDS diagnosis or mortality. This study may serve ARDS biomarker research in several ways. First it provides a novel manner to study different biomarker for the same clinical outcome. The calculation of the standardized difference of the means allows inclusion of various studies – even when absolute predictive values like sensitivity and specificity are not provided – and provides a measure to compare mutual performance. Second, this study provides a synthesis of ARDS plasma biomarker research performed in the last two decades. It not only demonstrates which plasma biomarkers have drawn more attention and which less, but also which plasma biomarkers perform better over various studies. The ranking provided in this study may therefore guide future biomarker research, both in decisions on prospective biomarker testing, and in composition of biomarker panels. Combining multiple biomarkers has previously been shown to enhance diagnostic accuracy [62,73].

In addition, the ranking of plasma biomarkers may elucidate the pathophysiology of ARDS. The biomarkers most strongly associated with ARDS diagnosis (Table 2) may well reflect lung tissue damage. Damage of the epithelial side of the alveolocapillary unit is reflected by release of KL-6 into lung interstitial lining fluid and the circulation [20,50,74], while inflammatory activation of the endothelial side is characterized by endothelial presentation of selectins on the cell surface, and release of proteins like soluble selectins, Ang-2 and vWF into the circulation [26,75,76]. The association of Ang-2 with ALI was confirmed in a very recent study [77]. Otherwise, among the five biomarkers most strongly associated with ARDS mortality, three were pro-inflammatory cytokines (IL-4, IL-2 and IL-1β), known to reflect systemic inflammation. It is conceivable, however, that severity of the underlying condition, e.g. sepsis, contributes to mortality independent of respiratory failure. Unfortunately, lack of data precluded analysis of these confounding effects. In terms of plasma biomarker performance, our study therefore indicates that ARDS diagnosis correlates with tissue damage, whereas ARDS mortality correlates more with markers of systemic inflammation.

Some considerations should be taken into account when evaluating the biomarker ranking provided in this study. Because not all studies provided absolute predictive values like sensitivity and specificity, the ranking in our study is based on OR. ORs may be difficult to interprete, and should be approached with care when applied to clinical practice [78]. Yet, for the goal of the current study, i.e. ranking of biomarker performance, the OR suffices and allows inclusion of a higher number of studies. Second, in this meta-analysis, only biomarkers addressed by multiple studies (≥2) were included. Due to this method, biomarkers evaluated in a single study, even though promising, are not considered, which may limit the view on plasma biomarker research

as a whole. Third, in the study of biomarkers for diagnostic utility, the choice of proper controls remains a matter of debate. One side of the discussion clings to the argument that critically ill patients at risk for ARDS (due to the presence of ARDS risk factors) form the proper controls, while the other side of the discussion clings to presence of edema, (resulting from other causes than increased permeability, c.q. hydrostatic edema) as the right control. A drawback of the latter is that patients with hydrostatic edema may not be at risk for ARDS per se, yielding the possibility that a biomarker predicts the risk factor for ARDS instead of ARDS. In the current study 7 (out of 29) of the studies used for the meta-analysis of biomarkers associated with ARDS diagnosis reported on critically ill patient with hydrostatic edema as controls [25,31,39,41,42,44,45]. Post-hoc analysis revealed that omission of these studies from the meta-analyses did not affect the magnitude or significance of the ORs presented in Table 1 (data not shown). Fourth, this meta-analysis considers the performance of ARDS biomarkers measured in plasma. A poor performance of a biomarker in the current study, however, does not exclude high performance of the same biomarker when measured in other compartments, like bronchoalveolar fluid (BAL) or exhaled gas, as recently shown for sLRP1 [79]. The reason to limit our research focus to plasma-derived biomarkers is that plasma-derived biomarkers have been studied most extensively, and that plasma sampling is often part of routine patient management. The fourth consideration is the question of biomarker reliability. The performance of a biomarker not only depends on its strength of association (for this study a high OR), but also on reproducibility of results [10]. The performance in the ranking of Tables 1 and 2 should therefore be weighed against the number of studies in which this pooled OR was observed. In this aspect, biomarkers like KL-6, vWF, RAGE, IL-8 and IL-6 (for ARDS diagnosis in the at risk population) and biomarkers like TNF-α and Il-8 (for ARDS mortality) can be considered most reliable, as relatively high pooled ORs were calculated from multiple studies.

This meta-analysis carries limitations. Since calculation of the OR was among others based on the exact P-value, studies with non-significant findings are more likely to drop out than studies with significant findings, which may lead to publication bias. This was the case in only 8 studies for 11 biomarkers [18,33,80-83]. To provide insight into the biomarkers and studies possibly involved we summarized these studies in Supplementary Table 6. Moreover, publication bias was evaluated using various statistical methods (Supplementary Table 5, Supplementary Figures 1 and 2). These tests evaluate the chance, but cannot fully exclude the possibility of publication bias. Although Egger's test was statistically significant in the meta-analysis of a number of biomarkers, adjustment for this possibility (by Duval & Tweedie's trim and fill) hardly affected the ORs of these biomarkers. Alternatively, a statistically significant Egger's test may result from true heterogeneity [84]. Several factors may contribute to heterogeneity: the various etiologies of

ARDS (Supplementary Table 2), different methodology for biomarker measurement, the relatively wide range of the interval between study inclusion and biomarker measurement (Supplementary Table 1), and the large time window in which included studies were published. During this period (1990-2011) changes in ARDS treatment and in biomarker assays may have altered biomarker performance. We identified a small number of studies that reported extreme ORs (OR < 0.10 or OR > 10.0) (Table 3). Although we could not identify a common denominator for the studies with extreme ORs, small sample size or age of the study may form an explanation. Despite these factors, no significant heterogeneity was found for biomarkers associated with ARDS diagnosis, whereas only mild heterogeneity was found for some biomarkers associated with ARDS mortality. Correction for the latter did not affect the ORs of these biomarkers (Table 3).

In conclusion, including 54 studies and 3753 patients this study provides an overview of the research performed after plasma-derived biomarkers for ARDS. The ranking of biomarkers according to their association with ARDS diagnosis in the at risk population or ARDS mortality, may help to improve ARDS diagnosis and outcome prediction.

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Chapter 7
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Supplementary Information

SUPPLEMENTARY METHODS

Search strategy

The search strategy was designed by three investigators (M.L.T., J.A. and A.B.J.G.) and further developed in collaboration with the institutions library staff. MEDLINE was searched using the following search entry: Biological Markers (mesh) or marker (tiab) or markers (tiab) or biomarker (tiab) AND Acute Lung Injury (mesh) or acute lung injur* (tiab) or acute pulmonary injur* (tiab) or ali (tiab) OR Respiratory Distress Syndrome, adult (mesh) or acute respiratory distress syndrome* or adult respiratory distress syndrome* (tiab) or shock lung* (tiab) or ards (tiab) AND blood chemical analysis (mesh: NoExp) or plasma (tiab) or blood (tiab) or serum (tiab) NOT (animals[mh] NOT humans[mh]). All MEDLINE articles from inception to January 1st 2012 were retrieved. In addition to the electronic search the personal bibliographic databases of all co-authors were evaluated.

Quality assessment of included studies

The QUADAS scoring was performed by two independent researchers (M.L.T. and J.A.). We evaluated different publications from the same group for reporting of one patient population in different publications. In case different studies reported on the same patient population, only the study with the largest patient population was used for meta-analysis.

SUPPLEMENTARY TABLES

Supplemental Table 1 – Study characteristics

Reference	Study population (n) (considered in current MA)	Male (%)	Age (years)	ARDS definition used	Moment of plasma sampling	Mortality (%)	QUADAS score
Agouridakis 2002 [48]	8	68	48 ¶	AECC + Fowler ‖	Within 2h of ICU admission	35 (*n.sp.*)	13
Aman 2011 [43]	83	79	60	AECC + LIS	Within 3h of ICU admission or 12h of sepsis diagnosis	13 (ICU mort)	13
Arif 2002 [44]	19	79	55 ¶	AECC	Within 72h of ICU admission	37 (ICU mort)	13
Bajwa 2009 [59]	177	55	62 ¶	AECC	Within 48h of ARDS diagnosis	40 (60-day mort)	14
Bauer 2000 [36]	66	75	60 ¶	AECC	Within 24h of study entry	61 (ICU mort)	14
Calfee 2008 [67] *	676	42	51	AECC	Upon admission	40 (180-day mort)	13
Calfee 2009 [42] *	67 †	51	51 ¶	AECC	Upon intubation	55 (hosp mort)	14
Chen 2009 [55]	88 †	88	74 ¶	AECC	Within 24h of ARDS diagnosis	61 (28-day mort)	13
Cheng 2003 [69]	38	50	43	AECC	Upon intubation	42 (28-day mort)	14
Chollet-Martin 1996 [34]	14	*n.p.*	61 ¶	LIS	Within 24h of intubation	55 (*n.sp.*)	13
Determann 2009 [18]	22	68	66 ¶	AECC	Upon initiation of mechanical ventilation	24 (*n.sp.*)	13
Determann 2010 [17]	36	61	59	AECC	Upon inclusion	25 (*n.sp.*)	12
Donnelly 1994 [29]	82	*n.p.*	50	LIS	Within 24h upon admission	50 (hosp mort)	13
Eisner 2003 [68] *	565	59	51	AECC	Upon admission	35 (180-day mort)	13
El Solh 2006 [40]	51	43	37 ¶	AECC	Within 8h upon intubation	6 (hosp mort)	14
Endo 2002 [35]	21	71	62 ¶	AECC	*n.p.*	*n.p.*	11
Fremont 2010 [25]	192	68	36 ¶	AECC	*n.p.*	10 (hosp mort)	14
Gallagher 2008 [53]	18	48	67	AECC	Upon admission	39 (*n.sp.*)	13
Ganter 2008 [52]	209	75	41	AECC	Within 0.5h of injury	13 (hosp mort)	13
Groeneveld 1995 [63]	13	46	53 ¶	Clinical criteria	Within 48h upon admission	39 (ICU mort)	13
Guervilly 2011 [56]	52	75	58	AECC	Within 24h upon ARDS diagosis	40 (28-day mort)	13
Headley 1997 [46]	34‡	65	44	Fowler	Within 24h upon ARDS diagosis	50 (ICU mort)	13
vd Heijden 2008 [26]	83	77	60 ¶	AECC + LIS	Within 24h upon study entry	12 (ICU mort)	13
Hensel 1998 [37]	17	60	63 ¶	LIS	Within 4h upon ICU admission	*n.p.*	13
Ishizaka 2004 [50]	33	77	68	AECC	Upon admission	32 (hosp mort)	14

Reference	Study population (n) (considered in current MA)	Male (%)	Age (years)	ARDS definition used	Moment of plasma sampling	Mortality (%)	QUADAS score
Jabaudon 2011 [30]	33	67	59 ¶	AECC	Upon admission	39 (28-day mort)	13
Kropski 2009[41]	23	48	40	AECC EF/plasma protein	Upon admission	56 (*n.sp.*)	13
Lee 2010 [32]	112	79	68	AECC	Within 24h upon admission	51 (*n.sp.*)	13
Leff 1993 [21]	26	*n.p.*	*n.p.*	Clinical criteria	Upon diagnosis of sepsis	35 (*n.sp.*)	12
Lesur 2006 [58]	75	61	63	AECC	Within 48h upon admission	38 (28-day mort)	12
Lin 2010 [54]	63	60	69	AECC	Upon admission	43 (28-day mort)	13
Matthay 2004 [70]	45	*n.p.*	42	AECC	*n.p.*	54 (hosp mort)	14
McClintock 2008 [61]	50	56	55	AECC	Within 48h upon ARDS diagnosis	42 (hosp mort)	13
Meduri 1995 [47]	21	59	47 ¶	Fowler criteria + LIS	Within 24h upon ARDS diagnosis	52 (ICU mort)	13
Moss 1995 [23]	29	72	46 ¶	Fowler criteria	Upon informed consent for study	*n.p.*	13
Nakamura 2011 [51]	20	60	65	AECC	Upon admission	50 (28-day mort)	13
Nakashima 2008 [49]	56	64	66	AECC	Within 24h of ARDS diagnosis	48 (60-day mort)	13
Nathani 2008 [19]	42	57	61 ¶	AECC	Upon admission	*n.p.*	12
Osaka 2011 [22]	27	48	77 ¶	Clinical criteria	Upon admission	0 (hosp mort)	13
Parsons 2005 [62] *	781	41	52	AECC	Within 24h upon study enrolment	*n.p.*	14
Prabhakaran 2003 [66]	25	52	44	AECC	0-12h after intubation	70 (hosp mort)	13
Roten 1990 [38]	50	62	49 ¶	Clinical criteria	Within 96h of ARDS onset	36 (*n.sp.*)	13
Rubin 1990 [24]	45	*n.p.*	*n.p.*	LIS	*n.p.*	51 (hosp mort)	13
Sakamaki 1995 [28]	19	42	49	Clinical criteria	Within 48h of ARDS diagnosis	43 (*n.sp.*)	12
Sato 2004 [20]	37	36	40	AECC	Upon admission	43 (*n.sp.*)	13
Siemiatkowski 2000 [27]	36	75	38 ¶	PEEP + LIS + static respiratory compliance	Upon admission	31 (*n.sp.*)	13
Takala 2001 [33]	11	63	50 ¶	AECC	Within 24h upon admission	23 (hosp mort)	13
Tseng 2008 [57]	22	82	63	AECC	Upon study enrolment	23 (14-day mort)	13
Uchida 2006 [31]	33	58	44 ¶	AECC	*n.p.*	52 (hosp mort)	12
Ware 2001 [65]	46	51	48	AECC + EF/plasma protein	Upon intubation	51 (hosp mort)	14

Reference	Study population (n) (considered in current MA)	Male (%)	Age (years)	ARDS definition used	Moment of plasma sampling	Mortality (%)	QUADAS score
Ware 2003 [45]	45	60	42	AECC + EF/plasma protein	Upon intubation	58 (hosp mort)	13
Ware 2004 [64] *	559	59	51	AECC	Within 24h upon study enrolment	35 (180-day mort)	14
Ware 2007 [39] *	878	59	54 ¶	AECC	Within 24h upon study enrolment	*n.p.*	13
Ware 2010 [60]	528	45	50	AECC	Upon study enrolment	27 (60-day mort)	13
Total	**3753**						**13 (11-14)**

* Studies indicated with an asterisk report results based on the same patient cohort. For calculation of the total number of patients, only the largest study on this patient cohort was taken into account. † Only the initial cohort was included here – the validation cohort was not included. ‡ Only the conventionally treated group was taken into account for the current study. ‖ Fowler et al. Am Rev Respir Dis 1985 [72]. ¶ Calculated with means of subgroups.
The number of patients provided under Study Population represents the number of patients used for meta-analysis, and may therefore be a subpopulation of the total study population. For age the mean or median is provided; if mean/median of whole cohort was not provided, a weighted average of the age in the subgroups was calculated. AECC = American European Consensus Conference; ARDS = Acute Respiratory Distress Syndrome; EF = oedema fluid; hosp = hospital; LIS = lung injury score; MA = meta-analysis; mort = mortality; n.p. = not provided; n.sp. = not specified; VAP = ventilator-associated pneumonia.

Suplemental Table 2 – ARDS etiology in included studies

Etiology ARDS	N (%)
Sepsis	1638 (44)
Pneumonia / Aspiration	2258 (60)
Trauma / Major surgery	857 (23)
Multiple transfusions	47 (1.3)
Pancreatitis	51 (1.4)
Other*	354 (9.4)
Not provided	450 (12)

Percentages were calculated taking 3753 as total. A single patient may have more than one risk factor for ARDS (e.g. pneumonia and sepsis).

* Hypovolemic shock, drug-related, near-drowning, toxemia of pregnancy, intracranial bleeding, sickle cell chest syndrome, disseminated cytomegalovirus infection, disseminated histoplasmosis and AIDS, vasculitic pulmonary hemorrhage, peritonitis, hypothermia, rhabdomyolysis, lung lymphoma, reperfusion edema after transplantation.

ARDS = Acute Respiratory Distress Syndrome.

Supplemental Table 3 – Weighted averages of biomarkers associated with ARDS diagnosis

Biomarker	At risk			ARDS		
	Weighted Mean ± SD	Range	Nr. of patients	Weighted Mean ± SD	Range	Nr. of patients
KL-6 (U/mL)	246 ± 20	220 – 279	51	452 ± 80	292 – 537	86
LDH (U/L)	171 ± 25	145 – 195	41	327 ± 55	274 – 380	12
vWF (% normal)	229 ± 102	106 – 435	220	329 ± 105	146 – 588	165
sP-selectin (ng/mL)	153 ± 41	139 – 272	76	368 ± 136	201 – 475	31
sRAGE (pg/mL)	1552 ± 1441	525 – 6400	144	4056 ± 4439	862 – 15500	173
sE-selectin (ng/mL)	19 ± 8	14 – 34	89	29 ± 16	19 – 53	20
Ang-2 (ng/mL)	3.4± 2.7	0.4 – 5.7	148	7.4 ± 2.7	1.3 – 8.6	127
IL-8 (pg/mL)	88 ± 74	16 – 175	164	140 ± 143	42 – 370	165
SP-D (ng/mL)	60 ± 20	17 – 83	114	106 ± 117	12 – 476	135
IL-6 (pg/mL)	229 ± 211	0 – 834	192	366 ± 201	220 – 712	215
IL-10 (pg/mL)	36.6 ± 5.2	32.0 – 42.4	153	89.3 ± 19.2	77.0 – 119.1	151
TNF-α (pg/mL)	16.0 ± 15.6	6.3 – 66.0	195	33.3 ± 32.8	7.5 – 127.0	239
PAI-1 (ng/mL)	65 ± 17	60 – 120	201	77 ± 13	73 – 138	920
IL-1β (pg/mL)	41.2 ± 20.8	3.0 – 52.4	88	42.5 ± 8.7	34.0 – 51.3	90
CRP (mg/dL)	12.0 ± 3.6	0.3 – 13.5	97	15.6 ± 6.6	0.2 – 18.6	59
CC-16 (ng/mL)	12.8 ± 3.4	7.7 – 22.0	114	19.4 ± 15.5	12.5 – 55.0	146
ICAM-1 ng/mL)	557 ± 166	177 – 629	101	628 ± 57	545 – 667	158
Transferrin (g/L)	1.3 ± 0.4	1.1 – 2.1	60	0.9 ± 0.08	0.8 – 1.0	25
sL-selectin (ng/mL)	1772 ± 521	262 – 1950	76	760 ± 230	479 – 940	23
Prot C (% normal)	71 ± 11	48 – 76	121	46 ± 2	37 – 47	824

Ang-2 = angiopoietin-2, ARDS = Acute Respiratory Distress Syndrome, CC-16 = clara cell protein-16, CRP = c-reactive protein, ICAM-1 = intercellular adhesion molecule-1, IL = interleukin, KL-6 = Krebs von den Lungen-6, LDH = lactate dehydrogenase, PAI-1 = plasminogen activator inhibitor-1, sE-selectin = soluble E-selectin, sL-selectin = soluble L-selectin, sP-selectin = soluble P-selectin, SP-D = surfactant protein-D, sRAGE = soluble receptor for advanced glycation end products, TNF-α = tumor necrosis factor-α, vWF = von Willebrand factor.

Supplemental Table 4 – Weighted averages of biomarkers associated with ARDS mortality

Biomarker	Survivors			Non-Survivors		
	Weighted Mean ± SD	Range	Nr. of patients	Weighted Mean ± SD	Range	Nr. of patients
IL-4 (pg/mL)	178 ± 38	151 – 230	26	246 ± 70	188 – 327	29
IL-2 (pg/mL)	245 ± 39	122 – 268	28	363 ± 122	303 – 651	34
KL-6 (U/mL)	298 ± 108	150 – 441	75	637 ± 261	380 – 1119	61
Ang-2 (ng/mL)	2.6 ± 0.7	2.5 – 5.3	194	7.6 ± 6.4	4.4 – 19.8	33
IL-1β (pg/mL)	105 ± 128	2 – 313	85	143 ± 207	3 – 518	123
TNF-α (pg/mL)	57.7 ± 76.1	0.1 – 198.0	119	91.6 ± 138.4	8.7 – 403.0	177
Procalcitonine (ng/mL)	4.3 ± 4.2	1.2 – 9.8	48	24.1 ± 41.1	4.4 – 106.7	26
CC-16 (ng/mL)	19.9 ± 0.03	19.9 – 20.0	57	67.8 ± 31.6	22.0 – 89.0	41
CRP (mg/dL)	14.8 ± 4.4	7.3 – 17.6	152	15.2 ± 2.9	13.4 – 20.2	132
IL-8 (pg/mL)	53 ± 69	33 – 414	998	178 ± 325	64 – 2938	535
vWF (% normal)	333 ± 43	106 – 370	778	442 ± 47	181 – 447	368
IL-10 (pg/mL)	14.3 ± 1.7	14.0 – 24.7	390	48.9 ± 37.3	34.0 – 141.9	247
PAI-1 (ng/mL)	61 ± 53	54 – 450	388	199 ± 249	111 – 900	161
ICAM-1 (ng/mL)	840 ± 137	338 – 1090	438	1073 ± 189	737 – 1500	191
IL-6 (pg/mL)	235 ± 96	69 – 934	953	460 ± 341	265 – 3347	527
SP-D (ng/mL)	83 ± 10	73 – 92	742	111 ± 12	101 – 125	339
sRAGE (pg/mL)	3630 ± 284	1797 – 3681	463	5145 ± 652	2231 – 5327	293
SP-A (ng/mL)	29.6 ± 1.8	22.0 – 30.0	392	30.6 ± 5.6	29.0 – 50.0	211
Protein C (% normal)	79 ± 14	31 – 95	456	57 ± 17	25 – 68	212

Ang-2 = angiopoietin-2, ARDS = Acute Respiratory Distress Syndrome, CC-16 = clara cell protein-16, CRP = c-reactive protein, ICAM-1 = intercellular adhesion molecule-1, IL = interleukin, KL-6 = Krebs von den Lungen-6, LDH = lactate dehydrogenase, PAI-1 = plasminogen activator inhibitor-1, PCT = procalcitonin, SP-A = surfactant protein-A, SP-D = surfactant protein-D, sRAGE = soluble receptor for advanced glycation end products, TNF-α = tumor necrosis factor-α, vWF = von Willebrand factor.

Supplemental Table 5 – Evaluation of publication bias

	Egger's regression		Duval & Tweedie's trim and fill			Orwin's fail-safe N
	Intercept	P-value	Original OR	Studies trimmed	Adjusted OR	Number of studies*
Biomarker Diagnosis						
KL-6	-2.8	0.72	6.06 [3.04 – 12.1]	0	6.06 [3.04 – 12.1]	36
vWF	1.1	0.49	3.06 [2.07 – 4.51]	1	2.84 [1.95 – 4.12]	21
sRAGE	1.5	0.39	2.92 [1.93 – 4.42]	2	2.35 [1.60 – 3.46]	19
IL-8	2.5	0.04	2.31 [1.54 – 3.47]	2	2.05 [1.38 – 3.03]	12
IL-6	1.6	0.17	2.15 [1.49 – 3.10]	2	1.94 [1.36 – 2.77]	15
TNF-α	3.0	0.05	1.97 [1.38 – 2.81]	2	1.63 [1.17 – 2.28]	11
Mortality						
KL-6	-2.6	0.57	4.48 [2.35 – 8.55]	0	4.48 [2.35 – 8.55]	23
IL-1β	6.9	0.07	2.92 [1.70 – 5.05]	1	2.30 [1.36 – 3.90]	20
TNF-α	5.0	0.05	2.89 [1.84 – 4.54]	0	2.89 [1.84 – 4.54]	23
IL-8	2.25	0.01	2.03 [1.67 – 2.47]	4	1.76 [1.46 – 2.12]	18
IL-6	2.98	0.02	1.98 [1.57 – 2.49]	1	1.92 [1.53 – 2.42]	9
Protein C	-2.5	0.11	0.51 [0.38 – 0.69]	0	0.51 [0.38 – 0.69]	9

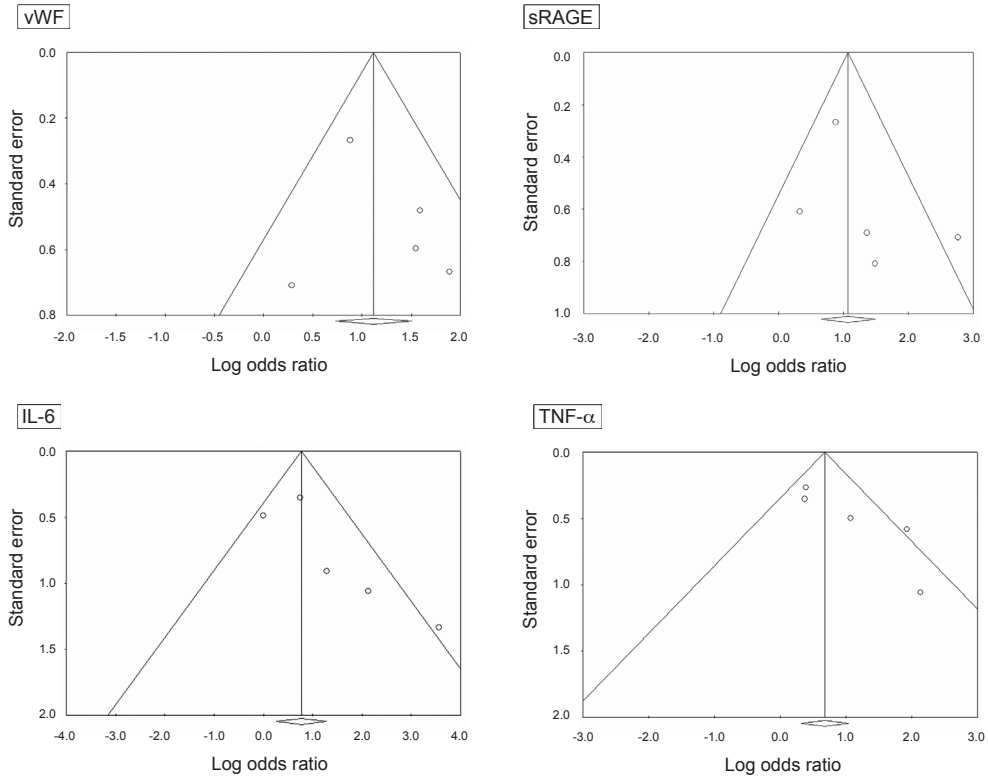
Evaluation of publication bias for the meta-analyses containing at least four studies. * Number of missing studies required to bring the odds ratio to a clinically trivial value (arbitrarily set at 1.25 for OR >1 or 0.8 for OR < 0.1), assuming that the missing studies find zero effect (OR of 1). IL = interleukin, KL-6 = Krebs von den Lungen-6, sRAGE = soluble receptor for advanced glycation end products, TNF-α = tumor necrosis factor-α, vWF = von Willebrand factor.

Supplemental Table 6 – Studies excluded because of insufficient statistical data

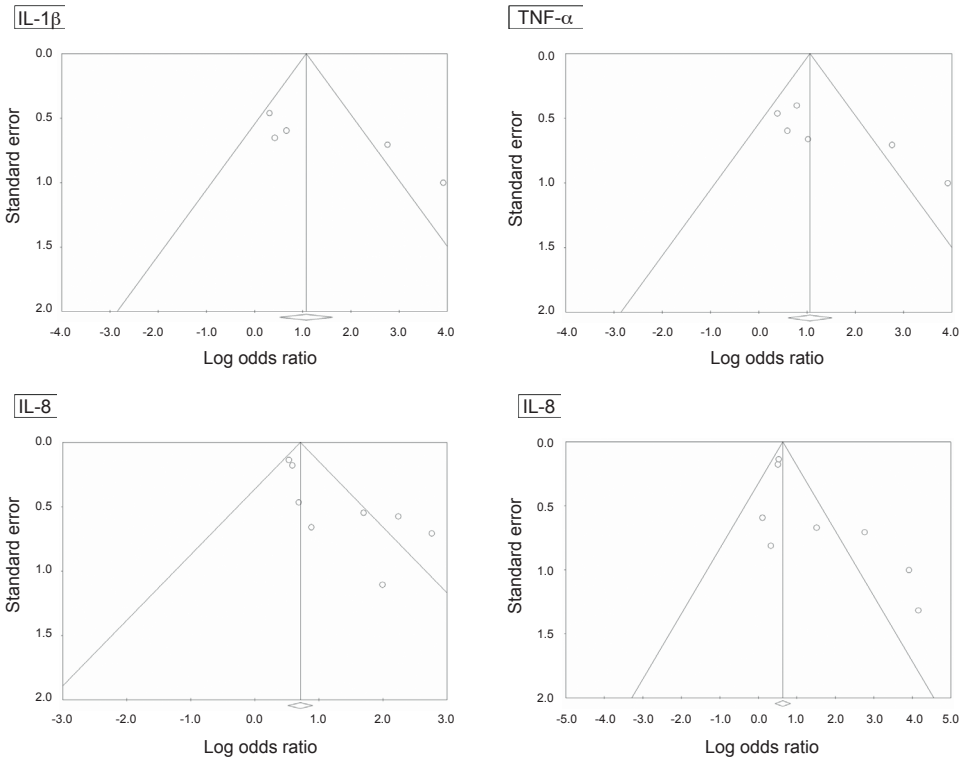
Outcome	Biomarker	Excluded studie
ARDS diagnosis	SP-A	Bersten 2001 [80]
	SP-D	Determann 2009 [18]
	vWF	Bajaj 1999 [81]
	IL-8	Amat 2000 [82]
	CRP	Schutte 1996 [83
	sE-selectin	Takala 2002 [33]
	PCT	Takala 2002 [33]
Mortality	SP-D	Cheng 2003 [69]
	TNF-α	Schutte 1996 [83]
	IL-6	Schutte 1996 [83]
	IL-8	Schutte 1996 [83]

Biomarkers for which one study was excluded because of missing P-values. ARDS = Acute Respiratory Distress Syndrome, CRP = c-reactive protein, IL-6 = interleukin-6, IL-8 = interleukin-8, PCT = procalcitonin, sE-selectin = soluble E-selectin, SP-A = surfactant protein-A, SP-D = surfactant protein-D, TNF-α = tumor necrosis factor-α, vWF = von Willebrand factor.

SUPPLEMENTARY FIGURES



Supplementary Figure 1 – Evaluation of publication bias in the prediction of ARDS diagnosis. Funnel plots of the meta-analyses presented in Figure 2. IL = interleukin, sRAGE = soluble receptor for advanced glycation endproducts, TNF-α = tumor necrosis factor-α, vWF = von Willebrand factor.



Supplementary Figure 2 – Evaluation of publication bias in the prediction of ARDS mortality. Funnel plots of the meta-analyses presented in Figure 3. IL = interleukin, TNF-α = tumor necrosis factor-α.